

Amendments to the Claims

The listing of claims will replace all prior versions, and listings of claims in the application.

Claim 1. (Currently amended) A method for producing embryoid bodies (EBs) from ~~multi-or~~ pluripotent cells comprising

(a) ~~agitation-of~~ agitating a liquid single cell suspension culture of ~~multi-or~~ pluripotent cells in a container ~~until generation-of~~ thereby generating cell aggregates, wherein said culture of pluripotent cells has a concentration of about 0.5×10^6 to 5×10^6 cells/ml; and

(b) ~~optionally~~ diluting the suspension, and further ~~agitation-of~~ agitating the suspension until formation of EBs.

Claim 2. (Original) The method of claim 1, wherein prior to step (a) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

Claim 3. (Currently amended) The method of claim 1 or 2, wherein said ~~multi-or~~ pluripotent cells are embryonic stem (ES) cells.

Claim 4. (Currently amended) The method of claim 3 ~~any one of claims 1 to 3~~, wherein said cells are ~~derived~~ obtained from a murine ES cell line.

Claim 5. (Currently amended) The method of claim 1 ~~any one of claims 1~~

~~to~~ 4, wherein the culture medium in step (a) ~~and/or~~ (b) or both is Iscove's Modified Dulbecco's Media (IMDM), 20 % fetal calf serum (FCS) and 5 % CO₂.

Claim 6. (Currently amended) The method of claim 1 ~~any one of claims 1 to 5~~, wherein the culture conditions in step (a) ~~and/or~~ (b) or both comprise 37 C and 95 % humidity.

Claim 7. (Currently amended) The method of claim 1 ~~any one of claims 1 to 6~~, wherein said culture of ~~multi-~~ or pluripotent cells has a concentration of about 1×10^6 to 5×10^6 cells/ml.

Claim 8. (Currently amended) The method of claim 7~~1~~, wherein the suspension in step (a) is cultured for about 6 hours.

Claim 9. (Currently amended) The method of claim ~~7~~ 8, wherein the suspension is cultured for about 16 to 20 hours.

Claim 10. (Currently amended) The method of any one of claims ~~7~~ 1, 8 ~~to~~ or 9, wherein the suspension in step (b) is cultured in T25 flasks.

Claim 11. (Currently amended) The method of claim 1 ~~any one of claims 1 to 10~~, wherein said dilution in step (b) is 1:10.

Claim 12. (Currently amended) The method of claim 1 ~~any one of claims 1 to 11~~, wherein the final concentration of EBs in the suspension culture is about 500 EBs/ml.

Claim 13. (Currently amended) The method of claim 1 ~~any one of claims 1 to 12~~, further comprising dividing the cell aggregates to the desired final concentration.

Claims 14-16. (Canceled)

Claim 17. (Currently amended) The method of claim 1 ~~any one of claims 1 to 16~~, further comprising culturing the EBs under conditions allowing differentiation of the EBs into ~~at least one cell type~~ cardiomyocytes.

Claim 18. (Canceled)

Claim 19. (Currently amended) The method of ~~any one of claims 1 to 16~~ claim 17, further comprising selection of ~~desired cell types~~ cardiomyocytes by use of one or more selectable markers ~~and/or agents~~ or both.

Claim 20. (Currently amended) The method of ~~any one of claims 1 to~~ claim 19, wherein said cell is genetically engineered.

Claim 21. (Currently amended) The method of ~~any one of claims 1 to~~ 19 or

20, wherein said cell comprises a selectable marker ~~and/or~~ a reporter gene or both.

Claim 22. (Currently amended) The method of ~~any one of claims 1 to~~ claim 21, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

Claim 23. (Original) The method of claim 22, wherein said selectable marker confers resistance to puromycin.

Claim 24. (Currently amended) The method of ~~any one of claims 1 to 23~~ claim 21, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

Claim 25. (Currently amended) The method of claim 24, wherein said cell type-specific regulatory sequence of the reporter gene is ~~substantially~~ the same as said cell type-specific regulatory sequence of the marker gene.

Claim 26. (Currently amended) The method of claim 25, wherein said reporter is ~~selected from different color versions of~~ enhanced green fluorescent protein (EGFP).

Claim 27. (Currently amended) The method of ~~any one of claims 22 to 26~~
claim 21, wherein said marker gene and said reporter gene are contained on the same
recombinant nucleic acid molecule.

Claim 28. (Original) The method of claim 27, wherein said marker gene and
said reporter gene are contained on the same cistron.

Claim 29. (Currently amended) The method of ~~any one of claims 22 to 28~~
claim 22, wherein said cell type-specific regulatory sequence is atrial- ~~and/or~~ ventricular-
specific.

Claim 30. (Currently amended) The method of claim 29, wherein said
regulatory sequence is a cardiac-specific regulatory sequence selected from promoters of
~~αMHC~~ alpha-myosin heavy chain (alpha-MHC) or ~~MLC2v~~ ventricular myosin light
chain 2 (MLC2v).

Claim 31. (Currently amended) An embryoid body obtained by the method
of claim 1 ~~any one of claims 1 to 30~~.

Claim 32. (Currently amended) A ~~differentiated cell~~ cardiomyocyte or tissue
~~of cardiomyocytes derived~~ obtained from the embryoid body of claim 31; ~~wherein said~~
~~cardiomyocytes are genetically engineered.~~

Claims 33-44. (Canceled)

Claim 45. (New) A method for producing embryoid bodies (EBs) from pluripotent cells comprising

(a) agitating a liquid single cell suspension culture of pluripotent cells in a container thereby generating cell aggregates, wherein said culture of pluripotent cells has a concentration of about 0.1×10^6 to 1×10^6 cells/ml; and

(b) agitating the suspension until formation of EBs.

Claim 46. (New) The method of claim 45, wherein prior to step (a) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

Claim 47. (New) The method of claim 45 or 46, wherein said pluripotent cells are embryonic stem (ES) cells.

Claim 48. (New) The method of claim 47, wherein said cells are obtained from a murine ES cell line.

Claim 49. (New) The method of claim 45, wherein the culture medium in step (a) or (b) or both is Iscove's Modified Dulbecco's Media (IMDM), 20 % fetal calf serum (FCS) and 5 % CO₂.

Claim 50. (New) The method of claim 45, wherein the culture conditions in

step (a) or (b) or both comprise 37°C and 95 % humidity.

Claim 51. (New) The method of claim 45, wherein said culture of pluripotent cells has a concentration of about 0.1×10^6 to 0.5×10^6 cells/ml.

Claim 52. (New) The method of claim 45, wherein the suspension is cultured for about 48 hours.

Claim 53. (New) The method of claim 45, further comprising diluting the resultant EBs to a concentration of about 100-2000 EBs/10 ml.

Claim 54. (New) The method of claim 45, further comprising culturing the EBs under conditions allowing differentiation of the EBs into cardiomyocytes.

Claim 55. (New) The method of claim 54, further comprising selection of cardiomyocytes by use of one or more selectable markers or agents or both.

Claim 56. (New) The method of claim 55, wherein said cell is genetically engineered.

Claim 57. (New) The method of claims 55 or 56, wherein said cell comprises a selectable marker or a reporter gene or both.

Claim 58. (New) The method of claim 57, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

Claim 59. (New) The method of claim 58, wherein said selectable marker confers resistance to puromycin.

Claim 60. (New) The method of claim 57, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

Claim 61. (New) The method of claim 60, wherein said cell type-specific regulatory sequence of the reporter gene is the same as said cell type-specific regulatory sequence of the marker gene.

Claim 62. (New) The method of claim 61, wherein said reporter is enhanced green fluorescent protein (EGFP).

Claim 63. (New) The method of claim 57, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.

Claim 64. (New) The method of claim 63, wherein said marker gene and said reporter gene are contained on the same cistron.

Claim 65. (New) The method of claim 58, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

Claim 66. (New) The method of claim 65, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from promoters of alpha-myosin heavy chain (alpha-MHC) or ventricular myosin light chain 2 (MLC2v).

Claim 67. (New) An embryoid body obtained by the method of claim 45.

Claim 68. (New) A cardiomyocyte or tissue of cardiomyocytes obtained from the embryoid body of claim 67.

Claim 69. (New) The method of claim 24, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

Claim 70. (New) The method of claim 60, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.